

71-23-72
075:73

Are Polyphosphates or Phosphate Esters Prebiotic Reagents?

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Received: 2 June 1995 / Accepted: 25 July 1995

Abstract. It is widely held that there was a phosphate compound in prebiotic chemistry that played the role of adenosine triphosphate and that the first living organisms had ribose-phosphate in the backbone of their genetic material. However, there are no known efficient prebiotic synthesis of high-energy phosphates or phosphate esters. We review the occurrence of phosphates in Nature, the efficiency of the volcanic synthesis of P_4O_{10} , the efficiency of polyphosphate synthesis by heating phosphate minerals under geological conditions, and the use of high-energy organic compounds such as cyanamide or hydrogen cyanide. These are shown to be inefficient processes especially when the hydrolysis of the polyphosphates is taken into account. For example, if a whole atmosphere of methane or carbon monoxide were converted to cyanide which somehow synthesized polyphosphates quantitatively, the polyphosphate concentration in the ocean would still have been insignificant. We also attempted to find more efficient high-energy polymerizing agents by spark discharge syntheses, but without success. There may still be undiscovered robust prebiotic syntheses of polyphosphates, or mechanisms for concentrating them, but we conclude that phosphate esters may not have been constituents of the first genetic material. Phosphoanhydrides are also unlikely as prebiotic energy sources.

Key words: Orthophosphate — Polyphosphates — Phosphorus pentoxide — Phosphate minerals — High-energy organic compounds — Thermal condensation

Introduction

Adenosine triphosphate (ATP) is used to drive biochemical reactions, and it is widely held that there was a prebiotic phosphate compound capable of similar functions. This is an attractive idea since it is easy to envision the evolution of biochemical pathways from being polyphosphate driven to being ATP driven. However, there is no known robust synthesis of polyphosphates or even pyrophosphate, thereby raising the question of whether polyphosphates were used in prebiotic reactions and indeed if the pre-RNA world had informational macromolecules that contained phosphate at all. Here we discuss previously reported prebiotic polyphosphate syntheses, some experiments of our own, and then the possible future directions of research in this area. There have been a number of reviews of prebiotic phosphorylations (Lohrmann and Orgel 1973; Miller and Orgel 1974; Hulshof and Ponnampuruma 1976) but there have been no reviews since then, and recent developments suggest that this topic deserves reconsideration.

It is important to distinguish between phosphate in the prebiotic world, the pre-RNA world, the RNA world, and the DNA/protein world. It is clear that phosphate was involved in the RNA world and the DNA/protein world if the RNA world is taken to mean that the genetic material had a ribose-phosphate backbone (and a deoxyribose-phosphate backbone in the DNA/protein world). The free energy carrier may have been ATP or polyphosphates (Kornberg 1995) or pyrophosphate (Baltscheffsky 1993). It is not clear what was involved in the pre-RNA world: The backbone may not have contained phosphate and the free energy source may not have contained phosphate. The same choices apply to the prebiotic world. We intend to show that phosphate is an unlikely reagent for

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the prebiotic world, and this may also apply to the pre-RNA world. But our comments do not apply to the RNA world and the DNA/protein world.

The Biological Occurrence of Phosphates

The phosphate group is central to modern biological systems. It most widely occurs as phosphate diesters in the genetic materials RNA and DNA, as phosphoanhydrides in the cellular energy currency ATP, and as phosphomonoesters in numerous metabolic intermediates. The properties of phosphate that enable it to fulfill these many functions have often been remarked upon (Westheimer 1987; Kornberg 1995). These authors have pointed out that phosphate is trivalent and trianionic and therefore able to form a link in a chain and still retain negative charge. They also point out that the phosphoanhydride bond is a high-energy bond, and yet the residual negative charge means that it is relatively stable in water. Molecules bearing phosphate groups are not soluble in lipid phases and can be retained in cells; the phosphate group is also able to act as a buffer and a chelator of metal ions.

Although the arguments for the fitness of phosphate in contemporary biological systems are rather convincing, even if they are constructed after the fact, this only shows that biological systems have evolved to use an ideal system. There is no guarantee that the first biological organisms used such a perfected system. We cannot simply extrapolate backward from modern organisms, as this makes no allowance for evolution, and even more important, prebiotic chemistry may not be able to activate phosphate except in very unusual environments. It could be said that amino acids, purines, pyrimidines, and, to a lesser extent, sugars fall out of prebiotic synthesis experiments in a robust manner, and therefore it seems likely that the metabolic pathways of the first organisms were similar to the modern ones and that activated phosphate groups were also widely utilized. It would be convenient if this were the case, but we shall show that it may not be possible.

The principal reason that phosphate has been invoked in prebiotic scenarios is because of the back extrapolation of modern metabolic pathways, an exercise for which there is no support as most modern metabolic pathways have no prebiotic counterpart at all. All of the proposed reasons for the presence of phosphate could equally well be applied to various organic functional groups. Amide bonds are relatively stable in water; carboxylate groups carry negative charge and also have chelating and buffering potential. Many unsaturated compounds, such as cyanide, contain potentially utilizable free energy. It should also be pointed out that condensed phosphates do not act as external sources of energy for modern organisms. Some bacteria have been shown to grow more rapidly in the presence of pyrophosphate (Wood 1985), and some others use photophosphorylation

to produce pyrophosphate rather than ATP (Baltscheffsky 1993). But in neither case has it been shown that the bacteria can use pyrophosphate as a source of energy.

Living organisms need a large amount of ATP to carry out biochemical processes. For example, humans produce approximately their own body weight of ATP per day. If the earliest organisms were to use ATP or a similar polyphosphate acquired from the environment as a source of energy, rather than as an internally produced mobile energy carrier, then they would have to assimilate similarly large amounts. Even if such large amounts were available in the environment, it is not clear whether this would be possible.

The Geological Absence of Polyphosphates

Except in living organisms and their products, polyphosphates are exceedingly rare on the modern Earth. No polyphosphate minerals have ever been discovered (Palache et al. 1951; Nriagu and Moore 1984) and there is only one known occurrence of a pyrophosphate mineral. This is a calcium pyrophosphate mineral of a few kilograms mass found in New Jersey (Rouse et al. 1988). However, there are low concentrations of dissolved polyphosphates (up to the tetramer) of volcanic origin that have been found in fumaroles by Yamagata et al. (1991). This source will be discussed below.

If it is to be claimed that polyphosphates were abundant on the early Earth, it is necessary to explain how the early Earth was different, and how such a difference could have resulted in the synthesis of polyphosphates. One difference of course was the absence of life. If present organisms were to use geologically produced polyphosphates, then this could account for their absence. There have been reports of bacteria which grow more rapidly in the presence of pyrophosphate (Wood 1985), but this does not appear to be a widespread phenomenon. In addition, organisms are likely to be a source of polyphosphates, at least in local areas, rather than a sink.

Another difference between the primitive Earth and the modern Earth is the presumed presence of high-energy organic compounds such as cyanoacetylene, cyanogen, and hydrogen cyanide. These compounds have high free energies of hydrolysis which could in theory be utilized in polymerization processes. These will also be discussed below.

Still another possible difference is the pH of the ocean or the type of phosphate mineral generally available. The pH of the ocean might have been different, but an explanation of how this could have happened is needed. A different phosphate mineral would also need an explanation. Hydroxy apatite, fluoro apatite, and other apatites are the predominant phosphate minerals on the Earth at present, with much, but not all, of it being precipitated by organisms. If apatite does not precipitate from sterile

solutions, then the situation might have been different. But so far no convincing case for this has been made.

High concentrations of magnesium ions inhibit the precipitation of apatite and it has been proposed that struvite ($\text{MgNH}_4\text{PO}_4 \cdot 4\text{H}_2\text{O}$) would have been the first phosphate mineral to have precipitated, rather than apatite (Martens and Harriss 1970; Handschuh and Orgel 1973). It is not clear whether the precipitation of apatite is slow enough on the geological time scale, in contrast to the laboratory time scale, so that sufficiently high concentrations could have accumulated. Another possibility is that the very insoluble ferric phosphate precipitated out, and was then reduced to the relatively soluble ferrous phosphate. This is a promising possibility since ferric is reduced to ferrous in the presence of organic matter and with ultraviolet light. This has not been reported as a phosphate concentration mechanism on the Earth today, but it may have been important in the past.

An important finding was made by Yamagata et al. (1991), who showed that acidic basalts containing apatite evolve phosphorus pentoxide (P_4O_{10}) when heated to $1,200^\circ\text{C}$. In addition they found approximately $5 \mu\text{M}$ concentrations of pyrophosphate and tripolyphosphate in a fumarole near Mt. Usu in Hokkaido, Japan. This is by far the most convincing case for a prebiotic source of polyphosphates.

The synthesis in basalts can be written



This is an example of an entropy-driven reaction where four molecules are converted to seven, so P_4O_{10} is stable at high temperature ($K_{1,000\text{K}} = 10^{11}$). However, orthophosphate becomes so stable at low temperatures ($K_{300\text{K}} = 10^{-62}$) that phosphorus pentoxide is the most powerful drying agent known.

There are two aspects to this synthesis that need to be understood to put it into a prebiotic context. First, does it proceed in most magmas or only in some examples? And secondly, how extensive could this synthesis have been on the prebiotic Earth? Let us assume that the yield of polyphosphate from orthophosphate is 100%. At the present time approximately 1 km^3 of lava is emitted per year ($0.1\% \text{ P}$, $\rho = 3 \text{ gcm}^{-3}$). So 10^{11} mol of phosphate as polyphosphate would be produced per year. If this was dissolved in the present ocean of 1.5×10^{21} l, then an increase in the condensed phosphate bond concentration of $7 \times 10^{-11} \text{ M}$ per year would result. Assuming a half-life for hydrolysis of 1,000 years (the rate in the absence of divalent cations at 0°C [Van Wazer 1958]) gives a steady-state concentration of $7 \times 10^{-8} \text{ M}$. This concentration is far too low to cause polymerization reactions to occur. Lava emissions are assumed to have been higher on the primitive Earth, perhaps by an order of magnitude, but this still would not affect our conclusions.

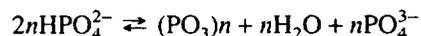
The above calculations are based on the best possible scenario. The yields for the volatilization of phosphorus

pentoxide from lava are not known. The half-life for hydrolysis of polyphosphates used were those measured in aqueous solution in the absence of divalent cations. These are known to catalyze the hydrolysis of polyphosphates and are present in relatively high concentrations in all natural waters. A more realistic yield of 1% and half-life of 10 years would reduce the steady-state concentration of phosphate as condensed phosphate to around 10^{-11} M . If polyphosphates were utilized close to their source, before they were dispersed throughout the ocean, then volcanic sources may have been sufficient. This, however, greatly reduces the area within which polyphosphates would have occurred and introduces the problem of the transport of organic molecules into these environments.

Can Polyphosphates Be Produced by Heating Rocks?

Although apatite is the predominant phosphate mineral on the Earth today, it has been claimed that apatite is a biologically produced mineral and that whitlockite is the kinetic and thermodynamically favored product under sterile conditions and therefore was the major phosphate mineral on the primitive Earth (Arrhenius et al. 1993).

Whitlockite contains monohydrogen phosphate (Mazghouni et al. 1981) and when heated produces ~10% pyrophosphate and smaller amounts of higher condensed phosphates (Sales et al. 1992). This heating to $250\text{--}550^\circ\text{C}$ took place in an open container so that the water could escape. We write the equation as



Hence the reaction is driven to the right by the escape of water. In the geological environment, such open systems can occur on beaches, lagoon margins, fumaroles, etc. However, the water cannot escape from buried and heated rocks. It has been pointed out by Byrappa (1983) that this is the reason that pyrophosphate and polyphosphate minerals do not occur in Nature: the trapped water in the rock would drive the reaction below to the left:



This was experimentally investigated in the case of neodymium orthophosphates. Polyphosphates will not form in such systems if the equilibrium partial pressure of water is above about 6 atm. This can be generated hydrostatically, lithostatically, or by heating to 500°C at constant volume. The value for calcium phosphate would not be much different from that observed for neodymium phosphate.

There is an additional factor not generally recognized. Polyphosphate solids, particularly calcium and magnesium salts, frequently hydrolyze on attempts to dissolve

Table 1. Some examples of the synthesis of phosphoanhydrides and phosphate esters using heat

Starting phosphate	Heating conditions	Product	Yield	Reference
NaH ₂ PO ₄	500–600°C	Cyclic trimetaphosphate	100%	Van Wazer 1958
NaH ₂ PO ₄ and KH ₂ PO ₄	200–600°C	Linear polyphosphates	100%	Van Wazer 1958
NaH ₂ PO ₄ and uridine	160°C	UMP	16%	Ponnamperuma and Mack 1965
Ca(HPO ₄) ₂ and uridine	160°C	UMP	11%	Ponnamperuma and Mack 1965
Na ₂ HPO ₄ and uridine	160°C	UMP	0.6%	Pannamperuma and Mack 1965
NaH ₂ PO ₄	160°C	Pyrophosphate	5–10%	Rabinowitz <i>et al.</i> 1968
NaH ₂ PO ₄	160°C	Triphosphosphate	1–5%	Rabinowitz <i>et al.</i> 1968
Na ₂ HPO ₄	160°C	Pyrophosphate	5–10%	Rabinowitz <i>et al.</i> 1968
Na ₂ HPO ₄	160°C	Triphosphosphate	1–5%	Rabinowitz <i>et al.</i> 1968
CaHPO ₄	160°C	Pyrophosphate	30–50%	Rabinowitz <i>et al.</i> 1968
CaHPO ₄	160°C	Triphosphosphate	10–30%	Rabinowitz <i>et al.</i> 1968
NH ₄ H ₂ PO ₄ and urea	85–100°C	Linear polyphosphates	60–100%	Österberg and Orgel 1972
Ca ₃ (PO ₄) ₂ , urea and uridine	85°C	UMP	30–80%	Handschuh <i>et al.</i> 1973
Na ₂ HPO ₄ , urea and thymidine	65°C	TMP	25%	Bishop <i>et al.</i> 1972
Ca ₃ (PO ₄) ₂ , (NH ₄) ₂ C ₂ O ₄ , and thymidine	90°C	TMP	15–30%	Schwartz <i>et al.</i> 1975
Ca ₃ (PO ₄) ₂ , (NH ₄) ₂ C ₂ O ₄ , and thymidine	90°C	Polyphosphates	1–50%	Schwartz <i>et al.</i> 1975
Trehalose	56°C	Trehalose phosphate	15%	Tarelli and Wheeler 1993
MgHPO ₄	250–550°C	Polyphosphates 2–10 mers	50%	Sales <i>et al.</i> 1992

them because of the strong crystal forces (Sales *et al.* 1993). This can be avoided in the laboratory by the use of chelating agents such as EDTA or oxalate that would not have been widely available on the primitive Earth.

If we assume that somehow this reverse reaction could be inhibited or if the water were somehow to escape or be removed, then a calculation similar to that for volcanic sources can be done. Approximately 1 km³ of sediment is weathered every year (0.1% P, $\rho = 3 \text{ g cm}^{-3}$). Assuming that all of the phosphorus in this is converted to polyphosphates, then the steady-state concentration of condensed phosphate bonds would have been the same as before, $7 \times 10^{-8} \text{ M}$. Even if we add this steady-state concentration to that which was volcanically produced, then the result, $1.4 \times 10^{-7} \text{ M}$, is also far too low to cause polymerization reactions to occur.

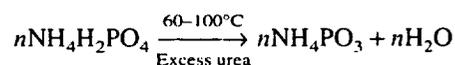
We are therefore skeptical that heated rocks were a source at all of polyphosphates because (1) the widespread occurrence of whitlockite is doubtful, (2) heating in a closed system does not give polyphosphates, and (3) an efficient mechanism is not available to concentrate them in a local area.

Thermal Syntheses of Polyphosphates

The synthesis of metaphosphates by heating dihydrogen phosphates (and pyrophosphates by heating monohydrogen phosphates) has long been known (Berzelius 1816; Van Wazer 1958). The temperatures used were generally 200–600°C. These were in open systems so that the water produced could escape. There are two problems in

applying this to the prebiotic environment. The first is that dihydrogen phosphate minerals are not known in Nature today. The second problem is how to heat dihydrogen phosphate minerals at 200–600°C in open systems. This could have occurred if the whole surface of the Earth was at this temperature, but that would have destroyed all of the organic compounds. Local areas at 200–600°C would have been extremely limited in extent.

There have been many attempts to produce polyphosphates at lower temperatures with very limited success (Table 1). One exception is the synthesis of ammonium metaphosphate from ammonium dihydrogen phosphate at the more reasonable temperatures of 60–100°C (Lohrmann and Orgel 1971; Österberg and Orgel 1972; Österberg *et al.* 1973; Handschuh *et al.* 1973). This gets around the high-temperature problem, but we are still left with the problem of a prebiotic source of the ammonium dihydrogen phosphate.



It is not clear how solid ammonium dihydrogen phosphate could have accumulated. It is very soluble but excess calcium ions would preferentially precipitate CaHPO₄ or Ca(H₂PO₄)₂ on a drying beach or lagoon margin. Large amounts of urea could have occurred, but only after concentration by evaporation. There is also the problem of dispersing the polyphosphates into the whole ocean. However, this may be circumvented if the ammonium metaphosphate was produced and used before being washed into the ocean.

Can Polyphosphates be Concentrated?

The polyphosphate problem could be overcome if the small amounts produced by volcanoes or other sources could have been concentrated in the critical area. The most obvious way of doing this is by the evaporation of a dilute solution of polyphosphates. This might work except that calcium ions are almost always more abundant than phosphate ions, and so the concentration is limited by the solubility of calcium polyphosphates (98 μM for calcium pyrophosphate, for example, Van Wazer, 1964). A chelating agent for calcium ions such as oxalate could have overcome this problem (Schwartz et al. 1975), but this would require oxalate to have been more abundant than the calcium ions, which is unlikely. Triphosphates are used in laundry detergent formulations to solubilize calcium ions by chelating them, which only works when the triphosphate is in excess concentration over the calcium ions. The same considerations apply to concentration by freezing.

Concentration of polyphosphates might have taken place close to a volcano or fumarole emitting phosphorus pentoxide, as was observed near Mt. Usu in Japan (Yamagata et al. 1991). Polyphosphate concentrations in these fumarole condensates were approximately micromolar. In addition, the resultant pools of water would have had a transient existence; the polyphosphates would hydrolyze, and a mechanism is needed to transport the organic compounds to be phosphorylated to these pools. Thus the areas where such concentration of polyphosphates could have taken place are so limited that they are of doubtful applicability.

Another possible process is to precipitate the polyphosphates and then solubilize them by removing the calcium or other metal ions. As discussed above, it is not likely that a calcium ion chelator would have been more abundant than the calcium ions. A related process is to somehow produce a massive deposit of calcium polyphosphates that either forms the bed of the lake or is a cliff at its side. This might work if the weathering was rapid enough, the concentration of calcium ions was low enough, and organic compounds were somehow supplied to the lake. This is highly improbable, owing to the multiplication of unlikely events (Shapiro 1986). This becomes even less likely if the continents had not yet formed.

A process related to precipitation is that of absorption onto a surface (e.g., of hydroxy apatite) or into a layered mineral (e.g., hydrotalcite). This would result in the concentration of the polyphosphates, and if organic compounds were also selectively absorbed, then there is the possibility of prebiotic phosphorylations taking place. There are a number of problems with this, such as whether the absorptions can take place from extremely dilute solutions (especially in the presence of chloride, carbonate, and other anions), the very limited area available, and how an adequate supply of dilute polyphosphate so-

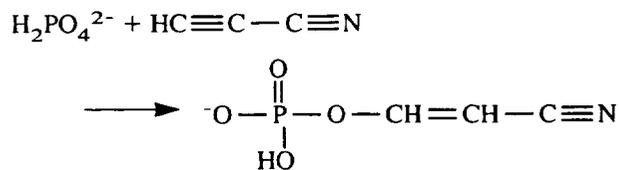
lution can pass through the mineral. For example, the interlayer region of hydrotalcite is $\sim 10 \text{ \AA}$ in phosphate at saturation, so the concentration of polyphosphates to this level from a 10^{-10} M solution would require 10^{11} units of solution per unit of hydrotalcite. This is a large amount of water to be extracted (100 km^3 of water per m^3 of hydrotalcite). If this were to come from rain percolating through a thermally metamorphosed phosphorite, assuming 1 m per year, it would take 10^{11} years of rainfall to saturate a layer of hydrotalcite 1 m thick, or 10^5 years for a layer 1 μm thick.

Chemical Polymerizations of Phosphate

There have been a large number of attempts to polymerize phosphates using organic compounds with high free energies of hydrolysis. Examples include cyanate, cyanogen, cyanoacetylene, cyanamide, and hydrogen cyanide. None of these syntheses can be considered robust.

Some of these high-energy compounds have also been used to polymerize amino acids, but we will not discuss these here, nor will we discuss polymerizations using polyphosphates. We will first discuss the synthesis of polyphosphates and ADP from high-energy organic compounds and then take up the use of such compounds to synthesize organic phosphates.

The number of prebiotic syntheses of pyrophosphate and polyphosphates from high-energy organic compounds is limited and a representative sample is shown in Table 2. The active reagent in the cyanate reactions is probably carbamyl phosphate which is formed reversibly from cyanate.



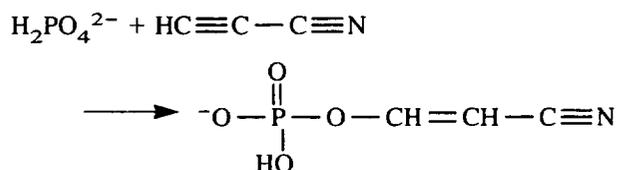
Cyanate and carbamyl phosphate have been produced in electric discharges (Yamagata and Mohri 1982) and from $\text{Fe}(\text{CN})_6^{3-}$ by ultraviolet light (Saygin 1981). Carbamyl phosphate will phosphorylate AMP and ADP non-enzymatically as well as produce acetyl phosphate (Saygin 1983). A number of reagents failed to produce pyrophosphate or polyphosphates. These include carbodiimides, dicyandiamide, and cyanogen (Beck and Orgel 1965), succinic anhydride, maleic anhydride, and carbon suboxide (Keefe and Miller 1996).

The prebiotic phosphorylations of organic compounds are more extensive. Many of these are listed in Table 3. The data in the table show that there are a variety of phosphorylations possible, but most of them are relatively low-yield reactions and use high concentrations of reagents. Another characteristic of the efficient reactions

Table 2. Some examples of the synthesis of phosphoanhydrides using high-energy organic compounds

Organic reagent	Phosphate	Product	Yield	Reference
Cyanate	Apatite	Pyrophosphate	26%	Miller and Parris 1964
Cyanate	Apatite	Pyrophosphate	1–2%	Beck and Orgel 1965
Cyanate and calcium phosphate	Tripolyphosphate	Trimetaphosphate	1%	Beck and Orgel 1965
Cyanate (Electric discharge)	Phosphate	Carbamyl phosphate	6%	Yamagata and Mohri 1982
Cyanate (Electric discharge)	Adenosine	AMP	2%	Yamagata <i>et al.</i> 1979, 1981
Carbamyl phosphate	AMP, ADP	ADP, ATP	2–17%	Saygin 1981
Carbamyl phosphate	Acetate	Acetyl phosphate	30%	Saygin 1983
Ferricyanide and visible light	Phosphate	Carbamyl phosphate	15%	Saygin 1981
Cyanamide	Phosphate	Pyrophosphate	?	Steinman <i>et al.</i> 1964
Dicyandiamide	Phosphate	Pyrophosphate	0.2–1.8%	Steinman <i>et al.</i> 1965
Dicyandiamide	ADP	ATP	<0.03% 0.5% (Kaolin)	Steinman <i>et al.</i> 1965
Dicyandiamide	Phosphate	Pyrophosphate	<0.1%	Beck and Orgel 1965
Carbodiimide	Phosphate	Pyrophosphate	<0.1%	Beck and Orgel 1965
Thioesters	Phosphate	Pyrophosphate	2–11%	Weber 1982
Diacetyl cysteamine and imidazole	Phosphate	Pyrophosphate and tripolyphosphate	3–8%	Weber 1981
Dithionite and oxygen	Phosphate	Pyrophosphate	0.24%	Gabel 1968
Cyanamide	dTMP	dTppdT	58%	Sherwood and Oró 1977
Ammonium formate	Phosphate	Pyrophosphate	53%	Keefe and Miller 1996
Thiocyanate and hydrogen peroxide	Phosphate	Pyrophosphate	26%	Keefe and Miller 1996
Maleic anhydride	Phosphate	Pyrophosphate	5%	Keefe and Miller 1996
Pantoyl lactone	Phosphate	Pyrophosphate	5%	Keefe and Miller 1996
Polymerizing HCN	Phosphate	Pyrophosphate	4%	Keefe and Miller 1996
Imidazole and calcium chloride	Phosphate	Pyrophosphate	4%	Keefe and Miller 1996

is that while the organic compound is very efficient in reaction with phosphate, the product is a poor or non-selective phosphorylating agent. The best example is cyanoacetylene (Ferris *et al.* 1970)



which reacts with phosphate at concentrations as low as 10^{-6} M, probably via a cyclic transition state, but the cyanovinyl phosphate is a poor phosphorylating agent.

There is one consideration that has not previously been taken into account: Even the most efficient high-energy carbon compounds cannot generate large amounts of polyphosphates. Let us assume that we start with 1 atm pressure of methane or carbon monoxide and assume that hydrogen cyanide is made quantitatively from the carbon source. This will give 36 mol cm^{-2} of hydrogen cyanide. If this is dissolved in an ocean of the present size and each hydrogen cyanide molecule gives rise to one condensed phosphate bond via the formation of a high-energy organic compound, then the concentra-

tion of condensed phosphate bonds would be 0.12 M. However, this production of hydrogen cyanide would take some time. Assuming that the hydrogen cyanide was produced over a period of one million years and that the half-life of the hydrolysis of pyrophosphate bonds is 1,000 years, the steady-state concentration of pyrophosphate bonds would be 1.2×10^{-4} M. It would be surprising if even 1% of this value were achievable. Keeping in mind that this is a very favorable case and hydrogen cyanide has not been shown to make polyphosphate bonds at all, the use of high-energy carbon compounds produced from atmospheric constituents does not seem promising. There are possible ways around this. The atmospheric carbon might be recirculated, the time periods of 1,000,000 and 1,000 years might be adjusted, and the high-energy compounds might be concentrated in a local area.

The Beilstein Experiment—An Attempt to Find a Prebiotic Compound Which can Condense Orthophosphate Efficiently

As discussed above, none of the high-energy compounds used to produce polyphosphates have proved particularly efficient. If some high-energy organic compound capable of polymerizing phosphate has been overlooked, it

Table 3. Some examples of the synthesis of phosphate esters using high energy organic compounds

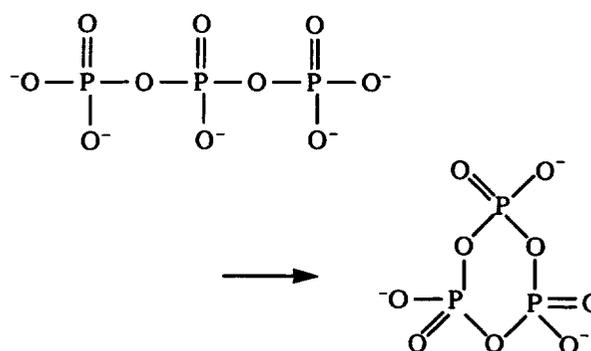
High energy compound	Reactant	Product	Yield	Reference
Cyanate (electric discharge)	Adenosine	AMP	0.02%	Yamagata <i>et al.</i> 1979, 1981
Cyanate	Uridine	UMP	1%	Lohrmann and Orgel 1968
Cyanamide (pH 2)	Glucose	Glucose-6-phosphate	1.5%	Steinman <i>et al.</i> 1964
Cyanamide	Ribose	Ribose-1-phosphate	8%	Halmann <i>et al.</i> 1969; Halmann and Schmidt 1970
Carbodiimide	Uridine	UMP	3%	Lohrmann and Orgel 1968
Dicyandiamide (pH 2)	Glucose	Glucose-6-phosphate	2.4%	Steinman <i>et al.</i> 1964
Dicyandiamide (pH 2)	Adenosine	AMP	?	Steinman <i>et al.</i> 1964
Dicyandiamide	Glucose	Glucose-6-phosphate	1.9%	Steinman <i>et al.</i> 1965
Cyanogen	Ribose	Ribose-1-phosphate	20%	Halmann <i>et al.</i> 1969; Halmann and Schmidt 1970
Cyanogen	Deoxyribose	Deoxyribose-1-phosphate	0%	Halmann <i>et al.</i> 1969; Halmann and Schmidt 1970
NCCONH ₂	Uridine	UMP	2%	Lohrmann and Orgel 1968
Ethylisocyanide	Uridine	UMP	1–10%	Lohrmann and Orgel 1968
Oxiranecarbonitrile	Phosphate	Glycoaldehyde phosphate	47%	Pitsch <i>et al.</i> 1994
Diiminosuccinonitrile	AMP	cAMP	18%	Ferris <i>et al.</i> 1984
Diiminosuccinonitrile or cyanogen bromide	Uridine	UMP	4%	Ferris <i>et al.</i> 1984
Cyanogen bromide	AMP 6-mer	AMP polymer	68%	Kanaya and Yanagawa 1986
<i>N</i> -cyanoimidazole	DNA 12-mer	DNA 24-mer	75%	Li and Nicolaou 1994
Carbodiimide	DNA 6-mer	DNA 12-mer	?	Sievers and von Kiedrowski 1994
Cyanamide	dTTP	dTMP oligomers	80%	Sherwood <i>et al.</i> 1977

should prove possible to detect such a compound by conducting a "Beilstein" (Prager and Jacobson 1918–1940) experiment. This is where almost all possible prebiotic organic compounds are synthesized simultaneously in an electric discharge (or other energy source) and allowed to react with an aqueous solution of orthophosphate. A 3-l flask with tungsten electrodes contained 100 ml of aqueous solution, 1 mM in sodium orthophosphate or tripolyphosphate and optionally 0.5 mM in CaCl₂ and 0.5 mM MgCl₂. The pH was adjusted to 8.2. The indicated gases were each 200 torr. The electric discharge was induced by a high-frequency 1-W Tesla coil. After 48 h the resulting solution was concentrated by evaporation to approximately 0.5 cm³, EDTA was added, and the yields of phosphorus products were determined by ³¹P nuclear magnetic resonance (NMR) at 121.5 MHz on a General Electric QE300 NMR spectrometer using phosphoric acid as an external reference.

The results of the discharge experiments are given in Table 4. The products were undetectable (<1%) using orthophosphate with or without calcium and magnesium ions. This experiment is more representative of a primitive Earth process than the usual experiments with high-energy compounds such as cyanate or cyanoacetylene in that the conditions are not so selective. Even so, we have used concentrations of orthophosphate far higher than are reasonable and still the yields were undetectable.

Because orthophosphate did not produce any product, we considered the possibility that a minor phosphate species could act as a high-energy phosphate by repeated recycling. For example, tripolyphosphate can be cyclized to trimetaphosphate, which is a phosphorylating agent, regenerating the tripolyphosphate as a product. Therefore, only a relatively small amount of tripolyphosphate would have to be synthesized by another route in the first

place. Table 4 also shows the yields with tripolyphosphate. Hexapolyphosphate was detected rather than cyclic trimetaphosphate because the trimetaphosphate produced apparently reacts with tripolyphosphate. The 20% yield of hexapolyphosphate (P₆O₁₉⁸⁻) with CO/N₂ is substantial, but it is not clear how efficient this reaction would have been on the prebiotic Earth. The yield becomes undetectable (<1%) on the addition of calcium and magnesium ions. The tripolyphosphate concentration in the experiment was much higher than it is reasonable to expect for the prebiotic Earth.



Conclusion

These results are in accord with a long line of prebiotic experiments. Pyrophosphate and polyphosphates can be synthesized under conditions considered to be prebiotic, but the yields are generally low and the conditions are rather forced. In particular the use of high concentrations of soluble orthophosphate (>10⁻³ M) cannot be considered prebiotic. The solubility of orthophosphate in the

Table 4. Percent yields of new condensed phosphates from electric discharges in various prebiotic atmospheres^a

Atmosphere	Percent yield of pyrophosphate from orthophosphate		Percent yield of hexapolyphosphate from tripolyphosphate	
	Na ⁺	Na ⁺ Ca ²⁺ Mg ²⁺	Na ⁺	Na ⁺ Ca ²⁺ Mg ²⁺
CH ₄ /H ₂ S/N ₂	0	0	12	0
CH ₄ /NH ₃	0	0	8	4
CH ₄ /N ₂	0	0	6	0
CO	0	0	0	0
CO/H ₂	0	0	0	0
CO/N ₂	0	0	20	0
CO ₂ /N ₂ /H ₂	0	0	0	0
CO ₂ /N ₂	0	0	0	0

^a The 3-l flask with tungsten electrodes contained 100 ml of aqueous solution, 1 mM in sodium orthophosphate or tripolyphosphate, and optionally 0.5 mM in CaCl₂ and 0.5 mM MgCl₂. The pH was adjusted to 8.2. The indicated gases were each 200 torr. The electric discharge was induced by a high-frequency 1-W Tesla coil. After 48 h the resulting solution was concentrated by evaporation to approximately 0.5 cm³. EDTA was added, and the yields of phosphorus products were determined by ³¹P NMR at 121.5 MHz on a General Electric QE300 NMR spectrometer using phosphoric acid as an external reference. Orthophosphate (singlet 5 to -1 ppm) is easily distinguished from pyrophosphate (singlet -6 to -12 ppm), the terminal phosphorus of tripolyphosphate (singlet -6 to -12 ppm), the central phosphorus of tripolyphosphate (triplet -18 to -22 ppm), trimetaphosphate (singlet -18 to -22 ppm), and hexapolyphosphate (multiplet -18 to -22 ppm). Hexapolyphosphate was detected rather than cyclic trimetaphosphate because the trimetaphosphate produced apparently reacts with tripolyphosphate

present ocean is 3×10^{-6} M, and as far as we are aware a natural water containing even 10^{-3} M orthophosphate has never been reported. The concentration of orthophosphate in the prebiotic ocean may have been higher if the pH was lower. But even this would not result in high concentrations of dissolved orthophosphate at reasonable pH values, and the hydrolysis of condensed phosphates under acidic conditions is much more rapid. Phosphorus is not an especially abundant element with crustal rocks containing on average only 0.09% (Vinogradov 1965). If all the phosphorus presently in the top 1 km of the crust was dissolved in the modern ocean the concentration would still only be 3×10^{-2} M.

One way around the problem would be to utilize the orthophosphate on the surface of apatite or other orthophosphate minerals. This so far has not proved to be particularly successful, although some positive results have been obtained (Miller and Parris 1964; Beck and Orgel 1965). Some phosphate mineral surfaces hydrolyze phosphate anhydrides such as ATP (Krane and Glimcher 1962). The addition of calcium and magnesium to our experiments quenched the pyrophosphate and hexapolyphosphate syntheses with few exceptions.

These results suggest that it may not be possible to produce adequate concentrations of high-energy phosphates using electric discharges or volcanic sources. We recognize that we may have missed some high energy compounds in these experiments so this statement needs to be taken with some reservation.¹

An alternative to the high local phosphate concentration hypothesis is to assume that phosphates did not play a part in the formation of the first living organism, either as a structural component of the pre-RNA molecules or as a free energy source. An example of an informational molecule with a nonphosphate backbone is the peptide nucleic acid system of Egholm et al. (1992). This mimics the structure of DNA quite well and indeed binds to DNA better than DNA does to itself. It is possible that the backbone for the first genetic material was not a polymer with a repeating unit. Thus the bases could have been held in place by a cyanide polymer or an acrolein amino acid polymer (Nelsestuen 1980). In addition there are models of the origin of life that do not involve phosphate, such as the clay theory of Cairns-Smith (1982) and the thioester world of de Duve (1991).

It may be more difficult to account for an alternative free energy source. The usual reagents, as discussed above, used to polymerize peptides or produce polyphosphates could be proposed, but their applicability would need to be investigated for the particular proposed prometabolic pathway or alternative genetic material. Another possibility is that the polymer has a negative free energy of formation from its precursors and therefore forms spontaneously. An example of this is the formation of polyglycine from glycine nitrile: this is thermodynamically favorable even at low concentrations, although it does not actually occur unless the glycine nitrile is very concentrated (Hanafusa and Akabori 1959; Losse and Anders 1961; Moser and Matthews 1968).

There may still be an undiscovered robust prebiotic synthesis of polyphosphates, or a mechanism of concentrating them, and we do not want to discourage further work in this area. In addition, it cannot be excluded that there are prebiotic processes requiring only small amounts of polyphosphates to take off and form self-replicating systems. However, we believe that alternative prebiotic free energy sources to polyphosphates deserve serious consideration and that searches in this area may be more fruitful than further attempts to find a prebiotic source of polyphosphates.

Acknowledgments. We thank the NASA Specialized Center of Research and Training (NSCORT) in exobiology at the University of

¹ It is perhaps significant that there have been few experiments in the last 20 years attempting to produce high-energy phosphates. This suggests that robust syntheses may not be possible.

California San Diego for a postdoctoral fellowship (A.D.K.) and grant support (S.L.M.).

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